



Contents lists available at ScienceDirect

## Journal of Food Composition and Analysis

journal homepage: [www.elsevier.com/locate/jfca](http://www.elsevier.com/locate/jfca)

Original research article

# Determination of arsenic species distribution in extra virgin olive oils from arsenic-endemic areas by dimensional chromatography and atomic spectroscopy

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## ARTICLE INFO

## Keywords:

Olive oil  
Arsenic species  
Liquid chromatography  
Atomic spectroscopy  
Food analysis  
Food composition

## ABSTRACT

Arsenic species were determined in extra virgin olive oils (EVOOs) by two dimension chromatography and atomic spectroscopy: inductively coupled plasma mass spectrometry (ICP MS) and electrothermal atomic absorption spectrometry (ETAAS). A first approach determined total As concentration in EVOOs samples by microwave assisted digestion and ICP MS from 2.01 to 152  $\mu\text{g kg}^{-1}$ . EVOOs with elevated As concentration were selected for protein extraction. In a first dimension, by size exclusion chromatography (SEC) coupled to ICP MS, a fraction of 66 kDa was identified and collected for analysis. Free and proteic As were separated by molecular weight cut off filters. Proteic and free arsenic concentrations determined by ETAAS range from 0.56–4.44 and 0.67–3.89  $\mu\text{g kg}^{-1}$  of As respectively. Finally in a second dimension, by anion exchange chromatography (AEC) coupled to ICP MS, dimethylarsenate and arsenite were determined in proteic fraction of EVOOs in concentrations of 1.68–2.86 and 0.53–0.55  $\mu\text{g kg}^{-1}$  respectively.

## 1. Introduction

Arsenic is a metalloid present in environmental and biological systems (soil, water and foodstuffs) (EPA, 2012). Arsenic is present in environment mostly as arsenite, arsenate and methylated forms generated by microorganisms metabolism (Jia et al., 2013; Zangi and Filella, 2012). The trivalent compounds of arsenic are thiol-reactive, and thereby inhibit enzymes or alter proteins by reacting with proteinaceous thiol groups. Pentavalent arsenate is an uncoupler of mitochondrial oxidative phosphorylation, by a mechanism likely related to competitive substitution (mimicry) of arsenate for inorganic phosphate in the formation of adenosine triphosphate (Casarett et al., 2001). In certain parts of Taiwan and South America, among others worldwide, the water contains high levels of this metalloid, and the inhabitants often suffer from dermal hyperkeratosis and hyperpigmentation (Hodgson, 2011). Contamination of shallow groundwater aquifers with As has been reported in over 20 countries around the world (Nordstrom, 2002). Many regions of Latin America are widely reported for the occurrence of high As in groundwater and surfacewater due to a combination of geological processes and/or anthropogenic activities, such as mining and smelting (Bhattacharya et al., 2006; Bundschuh et al., 2004). There are indications that the use

of As-contaminated water for irrigation has led to accumulations of As in surface soils which further lead to bio-accumulations of As in edible plants and crops (Rosas-Castor et al., 2014; Sadee et al., 2016). Livestock and humans may be exposed to As toxicity through plants and vegetables consumed (Khan et al., 2009).

The arsenic concentrations in the edible parts of crops depend on the availability of soil and the ability of a crop to take up As and translocate it to target organs (Zheng et al., 2011). In plants, the chelation phenomenon detoxifies arsenite through complexation with the thiol-rich peptide (Bluemlein et al., 2008; Castillo-Michel et al., 2011). Methylated forms of As have been found in plant tissues e.g. dimethylarsinate (DMA) and monomethylarsonate (MMA) (Huang et al., 2011; Ye et al., 2012). Generally, plants are less efficient at absorbing methylated species than inorganic As, but some plant species can accumulate higher concentrations of methylated As forms (Raab et al., 2007).

Determination of the chemical forms of arsenic in food (Corguinha et al., 2015; Jitaru et al., 2016) is critical because of the varying toxicity of different arsenospecies. Although the toxicity of the organoarsenicals is less than that of the inorganic As compounds (Liao et al., 2003; Nischwitz and Pergantis, 2006), the toxicity of organoarsenic species is of concern because of bioaccumulation in the organism (Kaise and

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<https://doi.org/10.1016/j.jfca.2017.12.011>

Received 15 February 2017; Received in revised form 2 August 2017; Accepted 7 December 2017

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Fukui, 1992). In this sense it is necessary to assess the levels of As in edible vegetable oils and to report possible contamination that would represent a health hazard (Zhuravlev et al., 2015).

A few researchers have made some progress on the determination of heavy metals in edible oils (Mendil et al., 2009; Sele et al., 2014). A novel approach, ultrasound-assisted dispersive liquid–liquid micro-extraction combined with liquid chromatography–mass spectrometry (UA-DLLME with LC–MS) has been reported (Wang et al., 2011). The results show that trace amounts of DMA were detected at concentration of  $6 \text{ ng g}^{-1}$  in frying oils. Chu et al. by means of inductively coupled plasma mass spectrometer (ICP MS) and ion chromatographic (IC) detector reported a method for speciation analysis of arsenic in edible oil (Chu and Jiang, 2011). However no arsenospecies were determined in unusual oils. Determination of arsenospecies in extra virgin olive oils (EVOOs) has not been reported before.

The present research describes a method for arsenic species determination in EVOOs from previously reported As endemic areas of Argentina (Bardach et al., 2015; Bhattacharya et al., 2006; Bundschuh et al., 2004). In a first approach a screening of As concentration in EVOOs was performed by microwave assisted digestion (MAD) followed by total As concentration determination by ICP MS. Since As shows high affinity for sulphidryl groups of proteins, this fraction was extracted, identified and isolated by size exclusion chromatography (SEC) in a first dimension. As distribution in proteic and non-proteic fractions of EVOOs was established by electrothermal atomic absorption spectrometry (ETAAS). Finally in a second dimension arsenospecies were determined in the proteic fraction, previous preconcentration with molecular weight cutoff filters (MWCO), by anion exchange chromatography (AEC). In both dimensions ICP MS was introduced for As monitoring. Arsenite, arsenate, MMA and DMA were determined for the first time in EVOOs.

## 2. Experimental

### 2.1. Reagents

Unless otherwise stated, the chemicals used were of analytical reagent grade and, therefore, no further purification was required. The standard  $\text{As}^{3+}$  solution ( $1000 \mu\text{g mL}^{-1}$ ) was prepared by dissolving  $0.3300 \text{ g}$  of  $\text{As}_2\text{O}_3$  (99.95% purity, Sigma, St. Louis, USA) in  $10 \text{ mL}$  of  $1 \text{ M}$  NaOH solution and diluting to a final volume of  $250 \text{ mL}$  with  $2 \text{ M}$  HCl. The standard  $\text{As}^{5+}$  solution ( $1000 \mu\text{g mL}^{-1}$ ) was prepared by dissolving  $0.4526 \text{ g}$  of  $\text{As}_2\text{O}_5 \cdot 2\text{H}_2\text{O}$  (99% purity, Aldrich, St. Louis, USA) in  $10 \text{ mL}$  of  $2 \text{ M}$  NaOH solution diluted to a final volume of  $250 \text{ mL}$  with  $2 \text{ M}$  HCl. Standards of monomethylarsonate and dimethylarsonate solution ( $1000 \mu\text{g mL}^{-1}$ ) containing  $543.21 \mu\text{g mL}^{-1}$  of As were prepared from analytical grade  $\text{CH}_3\text{AsO}(\text{ONa})_2 \cdot 6\text{H}_2\text{O}$  (99% purity, Merck, Darmstadt, Germany) (Torralba et al., 1994). Water, methanol (MeOH), *n*-hexane and acetone Optima LC–MS grade were purchased from Fisher Scientific (Fair Lawn, NJ, USA) as well as ammonium acetate. Ultrapure water ( $18 \text{ M}\Omega \text{ cm}$ ) was obtained from EASY pure (RF Barnstead, IA, USA). Sodium hydroxide was used provided by Biopack (Buenos Aires, Argentina) and nitric acid and hydrochloric acid, 65%, were provided by Sigma–Aldrich (St. Louis, USA).

### 2.2. Sample collection and treatment

Ten samples of EVOOs were obtained from olives processed before 24 h of harvest, and the process was carried out with the same equipment for all oils. Samples were collected from the most important EVOOs producing regions of Argentina. Details of procedence and origin are described in Table 1. The most important olive plants varieties grown corresponds to Arauco, Arbequina, Cornicabra and Frantoio. In this way, environmental and experimental parameters that could affect the content of the analytes under study are minimized. Olive oils correspond to *Olea europaea* L., subspecies Arauco,

**Table 1**  
Extra virgin olive oils characteristics and Arsenic concentrations determined.

	Procedence <sup>b</sup>	Variety	Total As ( $\mu\text{g kg}^{-1}$ ) <sup>a</sup>	$\text{As}^{3+}$ ( $\mu\text{g kg}^{-1}$ )	DMA ( $\mu\text{g kg}^{-1}$ )
1	Mendoza	Arbequina	$24.9 \pm 1.4$	ND <sup>d</sup>	ND <sup>e</sup>
2	Mendoza	Frantoio	ND <sup>c</sup>	ND <sup>d</sup>	ND <sup>e</sup>
3	Mendoza	Cornicabra	$6.87 \pm 0.2$	ND <sup>d</sup>	ND <sup>e</sup>
4	Mendoza	Arauco	$11.7 \pm 0.9$	ND <sup>d</sup>	ND <sup>e</sup>
5	Córdoba	Arbequina	ND <sup>c</sup>	ND <sup>d</sup>	ND <sup>e</sup>
6	Córdoba	Frantoio	$16.5 \pm 0.9$	ND <sup>d</sup>	ND <sup>e</sup>
7	Mendoza	Blend	$152 \pm 7.4$	$0.55 \pm 0.1$	$1.68 \pm 0.4$
8	San Luis	Arauco	$13.2 \pm 0.6$	ND <sup>d</sup>	ND <sup>e</sup>
9	La Rioja	Frantoio	$2.01 \pm 0.2$	ND <sup>d</sup>	ND <sup>e</sup>
10	La Rioja	Frantoio	$66.5 \pm 2.7$	$0.53 \pm 0.1$	$2860 \pm 0.5$

<sup>a</sup> Mean of 5 replicates.

<sup>b</sup> Argentinian regions.

<sup>c</sup> ND: no detected (Limit of detection:  $0.01 \mu\text{g kg}^{-1}$ ).

<sup>d</sup> ND: no detected (Limit of detection:  $0.00001 \mu\text{g kg}^{-1}$ ).

<sup>e</sup> ND: no detected (Limit of detection:  $0.00017 \mu\text{g kg}^{-1}$ ).

<sup>f</sup> DMA: dimethylarsonate.

Arbequina, Cornicabra and Empeltre.

MAD for total As determination was carried out in a microwave digester (Milestone, Sorisole, Italy). Digestion was performed according to the manufacturer indications:  $0.5 \text{ g}$  of olive oil samples were weighed and placed in individual microwave reactors. The aliquots were treated with  $7 \text{ mL}$  concentrated  $\text{HNO}_3$  and  $1 \text{ mL}$   $\text{H}_2\text{O}_2$ . Reactors were placed in the digester at a ramp temperature of  $10 \text{ min}$  up to  $200 \text{ }^\circ\text{C}$  and hold for 10 more minutes. The employed microwave power was up to  $1000 \text{ W}$ .

The method described by Martín-Hernández et al. (Martín-Hernández et al., 2008) was employed with modifications to extract proteins from EVOOs.  $10 \text{ mL}$  of cold *n*-hexane/acetone (1:1, v/v) ( $2 \text{ }^\circ\text{C}$ ) were added to  $5 \text{ g}$  of olive oil. The mixture was shaken vigorously, kept for  $1 \text{ h}$  at  $2 \text{ }^\circ\text{C}$ , and shaken every  $10 \text{ min}$ . The mixture was then centrifuged, and the supernatant was discarded. The precipitate was washed twice with  $1 \text{ mL}$  of cold *n*-hexane/acetone solution (1:1). After each washing, the mixture was centrifuged, and the supernatant was discarded. In both steps centrifugation lasted  $10 \text{ min}$  at  $7000 \text{ rpm}$  ( $6.026\text{g}$ ) at  $2 \text{ }^\circ\text{C}$  in a refrigerated centrifuge (Boeco U-320 R;Boeckel + Co (GmbH + Co), Hamburg, Germany). After the centrifugation stage, the supernatant was discarded and the pellet obtained was redissolved with water:methanol (80:20). This solution was centrifuged for  $5 \text{ min}$  at  $3500 \text{ rpm}$  ( $3.013\text{g}$ ), followed by freezing at  $-18 \text{ }^\circ\text{C}$  for  $1 \text{ h}$ . The remaining oil was kept frozen on the tube walls and a clear solution was obtained for analysis.

### 2.3. Size exclusion chromatography analysis

SEC was performed by coupling the chromatographer (Series 200, Perkin-Elmer (Thornhill, Canada) to ICP MS (Perkin-Elmer SCIEX, ELAN DRC-e; Thornhill, Canada). The argon gas with a minimum purity of 99.996% was supplied by Praxair (Córdoba, Argentina). Buffer ammonium acetate  $50 \text{ mM}$  was employed being adequate for coupling with ICP MS, since its volatility do not generate deposits on ICP cones. Bovine serum albumin ( $66 \text{ kDa}$ ), alcohol dehydrogenase ( $150 \text{ kDa}$ ),  $\beta$ -amilase ( $200 \text{ kDa}$ ), thyroglobulin ( $669 \text{ kDa}$ ), apoferritin ( $443 \text{ kDa}$ ) and equine myoglobin ( $17 \text{ kDa}$ ) were employed for calibration. Sulphur was monitored to investigate the presence of peptides and proteins, according to amino acids with sulphur residues like methionine and cysteine. The employed SEC column separates in a wide range from  $10$  to  $700 \text{ kDa}$ . This first dimension procedure, allowed the identification of As-S fractions correspondent with proteins and peptides molecular weight.

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